Report for 2004GU27B: Presence and Survival of Fecal Indicator Bacteria in Soil from the Banks of Major Rivers and Streams on Guam

There are no reported publications resulting from this project.

Report Follows

Project Title: Presence and Survival of Fecal Indicator Bacteria in Soil from the Banks of Major Rivers and Streams on Guam

Problem and Research Objectives

The use of fecal bacteria to monitor the hygienic quality of recreational waters has some serious limitations in many tropical and subtropical regions of the world. This is because favorable ambient temperatures encourage extended survival times of these organisms in the environment and dramatically increases the risk of false positives occurring. On Guam, *E. coli* and enterococci are used to monitor rivers and coastal waters around the island respectively. Both organisms have been observed to survive indefinitely in sediments and soils in Hawaii (Hardina and Fujioka 1991), Puerto Rico (Hazen 1988), southern Florida (Solo-Gabriele *et al*. 2000, Desmarais *et al.* 2002), and northern Australia (Davies *et al.* 1995). While the survival, growth and proliferation of *E. coli* and enterococci is suspected to occur in sediments and soils on Guam, the limited available data is inconclusive because it fails to differentiate between possible contributions from fecal and non-fecal sources. The fact remains, however, that exceedences of the recreational water quality standards are far more frequent during wet weather than they are during dry spells. This strongly suggests that local riverbank soil is a major reservoir for enterococci, and that these bacteria are mobilized into the coastal belt by erosive processes during prolonged periods of heavy rain.

The objectives of this study were to: a) confirm seasonal differences in recreational water quality exceedences on Guam from historical data, b) establish the presence of *E. coli* and enterococci in riverbank soil and c) determine whether or not *E. coli* and enterococci are capable of surviving in Guam soils over extended periods of time.

Methodology

To verify seasonal trends in local recreational water quality exceedences, weekly fecal indicator data sets for 39 coastal sites (Fig. 1) were reviewed for the period 1999-2003 (courtesy of Guam Environmental Protection Agency). Monthly water quality exceedences were subsequently plotted against the cumulative monthly rainfall recorded at Naval Air Station (NAS) in central Guam over the same time period.

To establish the presence of fecal indicator bacteria in soil, plugs of sediment were taken from a small holes excavated in the banks of the Pago and Sigua Rivers in central Guam. The Pago River receives drainage from the Ordot Landfill while the Sigua River is relatively unimpacted by the activities of man. Replicate soil samples were collected from both locations in sterile polypropylene syringes (25-ml) with the needle end cut off, 'cork-borer' fashion. They were then sealed in Whirl-Pak bags, chilled immediately on 'blue ice' and transported to the laboratory in insulated containers.

Approximately 5 g of ejected soil was shaken for 2-3 minutes with ~50 ml of sterile deionized water in sterile 100-ml polycarbonate bottles normally used for presence/absence testing. The soil suspensions were allowed to stand for 20 minutes to permit partial settlement of the clay fraction. A 10-ml volume of the clear surface layer was removed from each sample and made up to 100 ml with sterile deionized water.

Bacterial enumerations were made via the Quantitray™ method following addition of the appropriate growth media to the soil dilutions, i.e., Colilert® for *E. coli* and Enterolert® for enterococci. The samples were incubated at 35°C for 18h ± 2h for *E. coli* and 41°C for 24h ± 2h for enterococci. Confirmatory analysis was performed on 50-75% of all fluorescing wells. In such instances, samples were withdrawn into a sterile syringe from the back of the Quantitray™ pouch and streaked onto EMB agar and mE agar for *E. coli* and enterococci respectively.

The survival experiments are currently underway. We are also attempting to differentiate between fecal and non-fecal strains of both organisms using biochemical techniques in a manner similar to that described for *Enterococcus* spp. by Manero and Blanch (1999).

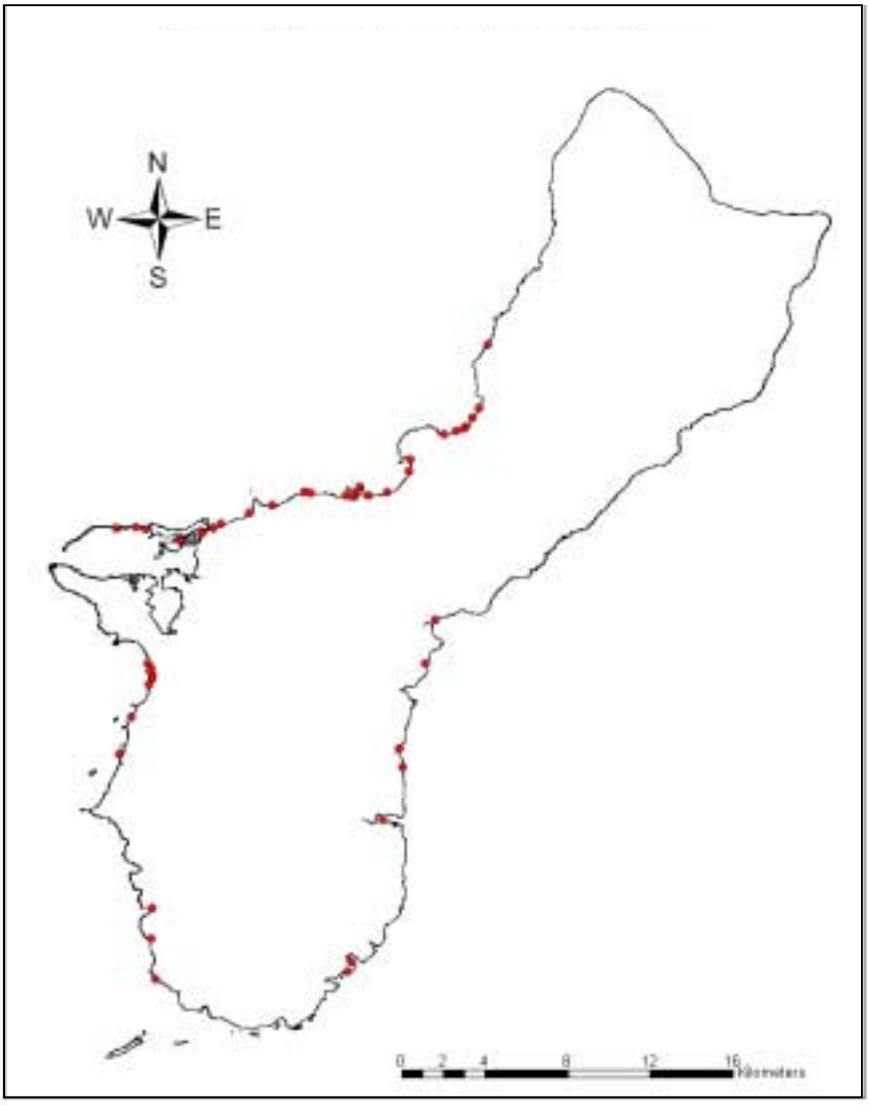

Figure 1: Map of Guam showing GEPA recreational water quality monitoring sites

Principal Findings and Significance

Exceedences of the USEPA recreational water quality standards on Guam are clearly related to storm events (Fig. 2) and predominantly occur during the wet season (July-December).

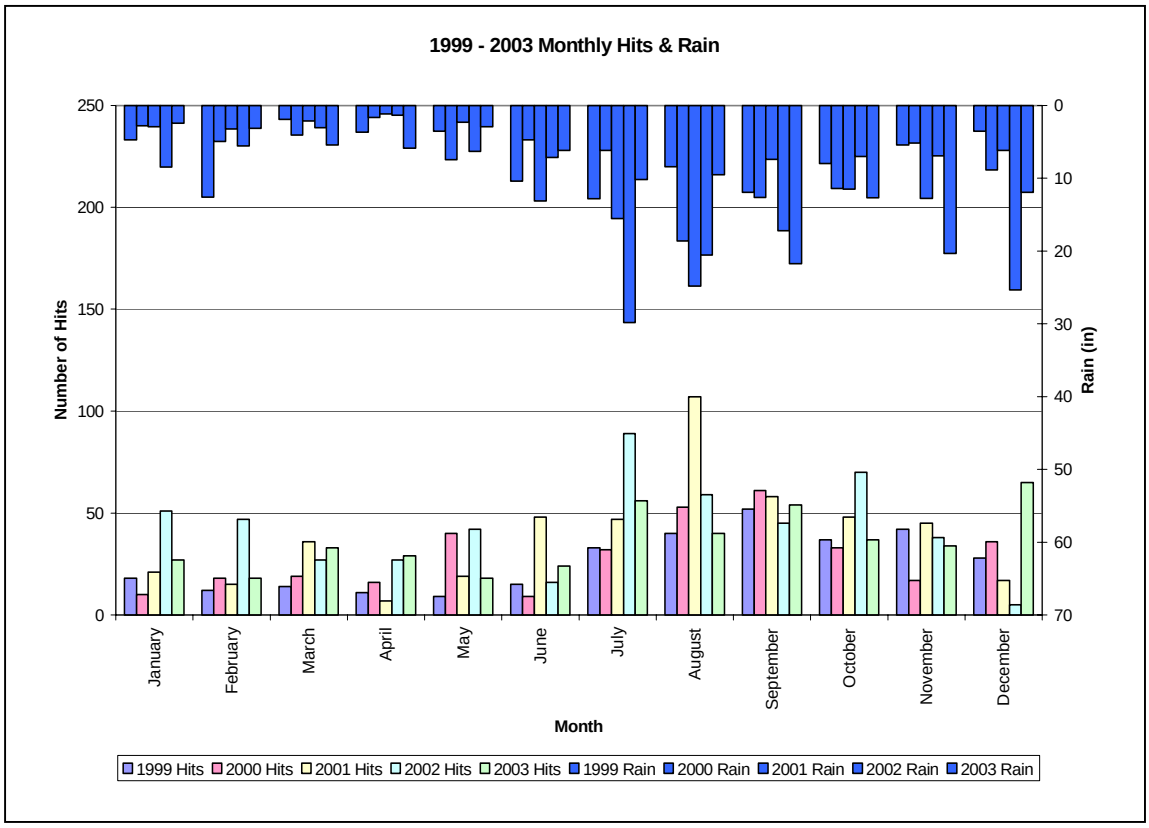

Figure 1: Cumulative monthly exceedences of the USEPA recreational water quality standards for the island of Guam (1999-2003) plotted against rainfall (blue bars) recorded at NAS, Tijan, Guam

The microbiological analysis of riverbank soil from the Pago and Sigua Rivers in central Guam are presented in Table 1. High counts of *E. coli* and enterococci were indicated in soil from both locations following incubation in Colilert® and Enterolert® growth media (Table 1). Differences between replicates were often highly variable and there was no clear relationship between bacterial densities and soil depth for either organism. The data for *E. coli*, however, suggest that horizontal distance from the river is a controlling factor. This may be related to variations in soil moisture content.

Confirmatory analysis consistently confirmed the presence of *E. coli* in all positive wells. In sharp contrast, only ~20% of well isolates confirmed for the presence of enterococci in Pago River samples and 0% confirmed in Sigua River samples. Clearly, there is a component in soil, other than enterococci, which causes fluorescence when incubated with the Enterolert® media. Whether this is removable by filtration is currently unknown.

Table 1: Fecal Indicator Bacteria in Soil from Pago and Sigua Rivers

River	Location	Depth	MPN Index/100 g soil	
			E. coli	Enterococci
Pago	River Bank			
	Site 1	Surface	525 - 2475	5211 - 9799
	Site 2	Surface	2659 - 6256	9697 - 17067
	Site 3	Surface	5929 - 6175	2784 - 9277
	Site 4	Surface	793 - 2854	4889 - 6009
Sigua	River Bank			
	Site 1	Surface	991	999 - 5971
	Site 1	5 cm	702 - 1535	2357 - 4518
	Site 1	10 cm	2020	438 - 1319
Sigua	I Meter Inland			
	Site 1	Surface	3941	2161 - 2617
	Site 1	5 cm	1081 - 1963	6830 - 8356
	Site 1	10 cm	613	1033 - 14213
Sigua	I0 Meters Inland			
	Site 1	Surface	<10	3897 - 10593
	Site 1	5 cm	264	2128 - 4839
	Site 1	10 cm	<10	234 - 523

Quantitray™ method using Colilert® and Enterolert® growth media for *Ecoli* and enterococcus respectively

Literature Cited

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